From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 15 May 2001 (15.05.01)	
International application No. PCT/FI00/00710	**Applicant's or agent's file reference** AP100055
International filing date (day/month/year) 22 August 2000 (22.08.00)	**Priority date** (day/month/year) 25 August 1999 (25.08.99)
Applicant AHOLA, Manja et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

12 February 2001 (12.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin d s Col mbettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Claudio Borton

Telephone No.: (41-22) 338.83.38

Copy for the Elected Office (EO/US) PCT/FI00/00710

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

TURUN PATENTTITOIMISTO OY
P.O. Box 99
FIN-20521 Turku
FINLANDE

Date of mailing (day/month/year) 12 February 2002 (12.02.02)	
Applicant's or agent's file reference AP100055	**IMPORTANT NOTIFICATION**
International application No. PCT/FI00/00710	**International filing date** (day/month/year) 22 August 2000 (22.08.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address

BIOXID OY
P.O. Box 114
FIN-20521 Turku
Finland

State of Nationality	State of Residence
FI	FI

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address

BIOXID OY
Tykistökatu 4 D, 4. krs
FIN-20520 Turku
Finland

State of Nationality	State of Residence
FI	FI

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Alexandre BOUVIER
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994) 004655758

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference AP 100055	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/FI00/00710	International filing date *(day/month/year)* 22.08.2000	Priority date *(day/month/year)* 25.08.1999
International Patent Classification (IPC) or national classification and IPC7 A61K 31/727, A61K 47/06, A61K 9/22		
Applicant BIOXID OY et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12.02.2001	27.11.2001
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Telex 17978 PATOREG-S Facsimile No. 08-667 72 88	Authorized officer Gerd Strandell/BS Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/FI00/00710

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☐ the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the claims:
pages _____, as originally filed
pages _____, as amended (together with any statement) under article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the drawings:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/FI00/00710

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-7	YES
	Claims		NO
Inventive step (IS)	Claims	1-7	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-7	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents are cited:

D1) WO 0050349 A2 (JOKINEN, MIKA ET AL), 31 August 2000 (31.08.00)

D2) WO 9745367 A1 (ORION-YHTYMÄ OY), 4 December 1997 (04.12.97)

D3) ART. CELLS, BLOOD SUBS., AND IMMOB. BIOTECH, Volume 26, No 4, 1998, Hongmei Chen et al, "Preparation and blood compatibility of new silica-chitosan hybrid biomaterials" page 431 - page 436

D4) STN International, File CA, Chemical Abstracts, volume 127, no. 10, 8September 1997 (Columbus, Ohio, US), Kim, Chulhee et al: "Heparin immobilization onto sol-gel derived organic-inorganic hybrid network", abstract no. 140503, & Surf. Modif. Polym. Biomater., (Proc. Am. Chem. Soc. Div. Polym. Chem. Int. Symp.) (1997, Meeting Date 1995, 157-164. Editors: Ratner, Buddy D. et al

D5) STN International, File CA, Chemical Abstracts, volume 128, 1997 (Columbus, Ohio, US), Negent, Helen M. et al: "Local drug delivery and tissue engineering regulate vascular injury"; abstract no. 145217, & Curr. Pharm. Des. (1997), 3(6), 529-544

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

Document D1) was published later than the international filing date and later than the priority date claimed. Therefor, in the International Search Report the category for this document is corrected to "E, X" instead of "P, X".

Document D2) discloses a controllably dissolvable silica-xerogel prepared via sol-gel process and containing a biologically active agent, such as a medicine, a protein, a hormone, etc.. The document does not mention heparin or related biologically active acidic polysaccharides as biologically active agents. Thus, the claimed invention is novel. The chemical properties, such as steric, electronic and hydrophobic characteristics, of polysaccharides differ highly from the agents disclosed in D2). Thus, it is not evident that what is disclosed in D2) would be applicable to heparin or related biologically active acidic polysaccharides.

Document D3) discloses silica-chitosan hybrid biomaterials produced by using biopolymer chitosan and its heparin-like derivative as the organic species to be incorporated into the silicon (TEOS) based network.

Document D4) discloses a process of preparing matrix-immobilised heparin. Butanediol was condensed with 3-isocyanatopropyltriethoxysilane and subjected to a hydrolytic polymerisation sol-gel process and then treated with heparin.

Document D5) discloses the controlled release of heparin from polymeric matrixes, whereas the present invention pertains to the controlled release of heparin from a sol-gel derived silica xerogel. The chemical structure of silica xerogels differs significantly from polymeric matrixes. The difference in, e.g. chemical and physical, structures causes different release behaviour of heparin from these materials.

None of the cited documents D2) to D5), taken alone or in combination, explicitly discloses the claimed composition or process. It must also be noted that the xerogel of the present invention is derived from a tetraalkoxysilane. According to the invention part of said tetraalkoxysilane is replaced with an organomodified alkoxysilane, which replacement is not disclosed by any of the cited references. Furthermore, the composition obtained has a high release rate of heparin. The achieved result is not obvious to a person skilled in the art. Consequently, the claimed invention is novel and is considered to fulfil the requirements of inventive step and industrial applicability.

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
WO 0050349	31.08.2000	21.02.2000	22.02.1999

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
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International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/FI00/00710	22.08.2000	25.08.1999
International Patent Classification (IPC) or national classification and IPC7		
A61K 31/727, A61K 47/06, A61K 9/22		
Applicant		
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- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
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Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Telex 17978 PATOREG-S Facsimile No. 08-667 72 88	Authorized officer Gerd Strandell/BS Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1998)

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International application No.
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/[redacted]100/00710

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	Claims		NO
Industrial applicability (IA)	Claims	1-7	YES
	Claims		NO

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D5) STN International, File CA, Chemical Abstracts, volume 128, 1997 (Columbus, Ohio, US), Negent, Helen M. et al: "Local drug delivery and tissue engineering regulate vascular injury"; abstract no. 145217, & Curr. Pharm. Des. (1997), 3(6), 529-544

.../...

Supplemental Box
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Continuation of: V

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VI. Certain documents cited

1. Certain published documents (Rule 70.10)

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WO 0050349	31.08.2000	21.02.2000	22.02.1999

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 March 2001 (01.03.2001)

PCT

(10) International Publication Number
WO 01/13924 A1

(51) International Patent Classification[7]: A61K 31/727, 47/06, 9/22

(21) International Application Number: PCT/FI00/00710

(22) International Filing Date: 22 August 2000 (22.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
19991806 25 August 1999 (25.08.1999) FI

(71) Applicant *(for all designated States except US)*: **BIOXID OY** [FI/FI]; P.O. Box 114, FIN-20521 Turku (FI).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **AHOLA, Manja** [FI/FI]; Iltatähdentie 4 as 91, FIN-20200 Turku (FI). **SÄILYNOJA, Eija** [FI/FI]; Käpytie 2 B 48, FIN-20810 Turku (FI). **SALONEN, Jukka** [FI/FI]; Puolalanpuisto 4 B A 4, FIN-20100 Turku (FI). **PENTTINEN, Risto** [FI/FI]; Lehmustie 3, FIN-20720 Turku (FI). **YLI-URPO, Antti** [FI/FI]; Värttinäkatu 17, FIN-20660 Littoinen (FI).

(74) Agent: **TURUN PATENTTITOIMISTO OY**; P.O. Box 99, FIN-20521 Turku (FI).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/13924 A1

(54) Title: NOVEL COMPOSITIONS FOR CONTROLLED RELEASE OF A BIOLOGICALLY ACTIVE AGENT, AND THE PREPARATION THEREOF

(57) Abstract: This invention relates to a composition for controlled release of a biologically active agent from a carrier. The biologically active agent is heparin or a related biologically active acidic polysaccharide and the carrier is a sol-gel derived silica xerogel. The xerogel is derived from a tetraalkoxysilane such as tetrethoxysilane (TEOS) and part of the tetraalkoxysilane is preplaced by an organomodified alkoxysilane, preferably an alkylsubstituted alkoxysilane. The invention also concerns a method for the preparation of said composition.

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JC13 Rec'd PCT/PTO 22 FEB 2002

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NOVEL COMPOSITIONS FOR CONTROLLED RELEASE OF A BIOLOGICALLY ACTIVE AGENT, AND THE PREPARATION THEREOF

This invention concerns a composition for the controlled release of a biologically active agent from a carrier, and the preparation of said composition.

BACKGROUND OF THE INVENTION

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference.

By xerogel is meant a dried gel. Silica xerogels are partly hydrolyzed oxides of silicium. Hydrolyzed oxide gels can be produced by a sol-gel process, which has been used for producing ceramic and glass materials for several years.

The sol-gel process is based on hydrolyzation of a metal-alkoxide and subsequent polymerization of the metal hydroxides as follows:

1) $Si(OR)_4 + H_2O$ ----> $HO\text{-}Si(OR)_3 + ROH$

2) $HO\text{-}Si(OR)_3 + 3\ H_2O + ROH$ ------> $Si(OH)_4 + 4\ ROH$

3) $Si(OH)_4 + Si(OH)_4$ -------> $(HO)_3Si\text{-}O\text{-}Si(OH)_3 + H_2O$

As the polymerization reaction progresses, additional chains, rings and three-dimensional networks are formed, and a gel, comprising water, the alcohol of the alkoxy group and the gel itself, is formed. The sol may also contain other additives, such as acids or bases, which are used as catalysts for the reaction. Further additives

thrombus formation and impaired function or occlusion of medical devices. Intravascular stenting is often used after angioplasty to prevent a reocclusion of the damaged vessel following dilatation. One problem inherent to stent implantation is a possible restenosis. The process of restenosis is attributed to myointimal hyperplasia as well as to thrombus formation (Palmaz, 1993, Van Beusekom et al., 1993). The interaction of platelets with the stent surface may have significance not only due to their involvement in thrombus formation, but also by the release of platelet derived growth factor that may be included in the stimulation of smooth muscle cell growth (Palmaz, 1993, Ross, 1986). Heparin is routinely used for the prophylaxis of both surgical and medical thrombosis.

However, there is no disclosure or suggestion in prior art indicating that compositions for the controlled release of heparin could be achieved by incorporating heparin in a sol-gel derived silica xerogel, and that such a composition would be useful for treating and/or preventing thrombosis. Known heparin preparations are administered as injections. Thus, there is a great need for more convenient administration routes of heparin, especially for long acting, controlled release dosage forms of heparin.

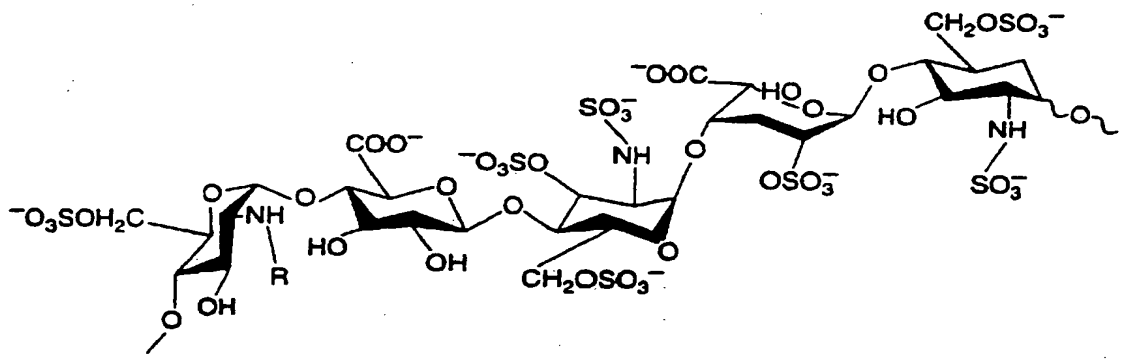
OBJECTS AND SUMMARY OF THE INVENTION

The aim of this invention is to provide a composition for the controlled release of heparin or a related biologically active acidic polysaccharide , wherein said composition can be used for systemic or local prophylaxis and/or treatment of medical or surgical thrombosis.

Another object is to provide a method for the preparation of a composition for the controlled release of heparin or a related biologically active acidic polysaccharide .

DETAILED DESCRIPTION OF THE INVENTION

Heparin is a linear polysaccharide containing repeated units of six sugar residues, each consisting of an alternating sequence of sulfate derivatives on N-acetyl-D-glucosamine and D-iduronate (formula I). Heparin is a powerful anticoagulant and it is also a component of the extracellular matrix of blood vessels and promotes endothelial cell growth *in vitro*.



(I)

According to this invention, heparin can alternatively be replaced by a related biologically active acidic polysaccharide. As examples of such acidic polysaccharides having antithrombotic effects can be mentioned heparan sulfate proteoglycan, sulfonated hyaluronic acid and the like.

The heparin or the related biologically active acidic polysaccharide can either be of natural origin or biotechnically manufactured.

The purpose of the present study was to evaluate the suitability of sol-gel produced silica xerogel as the carrier matrix for controlled release of heparin or a related acidic polysaccharide. The influence of sol-gel parameters, such as catalysts or various alkoxysiloxanes, and the effect of heparin concentration were studied. Also the maintenance of biological activity of the drug after sol-gel process was tested. The release of heparin was linear according to zero order kinetics, and the release

The invention will be described more in detail in the Experimental section in the following non-limiting examples.

Experimental

Preparation of silica sol

The silica sol loaded with heparin was prepared by a two step sol-gel process using acid as a catalyst (Ellerby *et al.* 1992). The following reagents were used, tetraethoxysilane (TEOS) (Aldrich), deionized water, nitric acid (HNO_3) (Merck), acetic acid (CH_3COOH) (Merck), ammonium hydroxide (NH_4OH) (Merck)) and heparin sodium salt (Orion Corporation, Finland). The biological activity of the used heparin was 84 IU/mg measured by Factor Xa assay (HEPRN). The first step of the reaction series was a hydrolysis reaction between water and alkoxide. The mol ratio of the silica sol was $TEOS:H_2O:HNO_3 = 1 : 15 : 0.0015$ and $TEOS:H_2O:CH_3COOH = 1 : 15 : 0.026$, respectively. Modification of the nitric acid catalyzed sol was carried out by co-hydrolysis of TEOS with the following organomodified (i.e. alkylsubstituted) alkoxysilanes: dimethyldiethoxysilane $Me_2Si(OEt)_2$ (DMDES) (Lancaster), methyltriethoxysilane $MeSi(OEt)_3$(METES) (Aldrich) or ethyltriethoxysilane $EtSi(OEt)_3$ (ETES) (Lancaster). For the partial substitution of TEOS, 10 or 25 mol-% organomodified alkoxysilane was used. After the first step, i.e. hydrolysis reaction, pH was raised to 4.5-4.8 with base (0.1 or 1 M NH_4OH) before heparin addition. The heparin sodium salt was first dissolved in the deionized water and then added to the hydrolysis solution. The concentration of heparin in silica sol ranged from 1 wt % to 4 wt % calculated on the sol, corresponding to 6.8 - 29.2 wt % in the air dried silica xerogel. The silica sol was cast into Blister plate wells, kept at 40°C and 40% relative humidity for polycondensation and ageing. The aged silica gels were dried at 40°C and 40% relative humidity to constant weight to obtain silica xerogels containing incorporated heparin. The formulations prepared are disclosed in Table 1.

In vitro **release experiments**

Dissolution test

The dissolution profiles of heparin and silica from the silica xerogel matrixes were studied in a shaking water bath at 37 °C. Simulated body fluid (SBF) was used as a dissolution medium. SBF was prepared by dissolving reagent grade NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \times 3H_2O$, $MgCl_2 \times 6H_2O$, $CaCl_2 \times 2H_2O$, Na_2SO_4 in deionized water (Table 2). The solution was buffered with tris(hydroxymethyl)aminomethane (TRIZMA) and hydrochloride acid (HCl) at physiological pH 7.40. The composition of inorganic ions emulated that of human blood plasma.

Table 2

Reagent	concentration (mM)
NaCl	136.8
$NaHCO_3$	4.2
KCl	3.0
$K_2HPO_4 \times 3H_2O$	1.0
$MgCl_2 \times 6H_2O$	1.5
$CaCl_2 \times 2 H_2O$	2.5
Na_2SO_4	0.5
TRIZMA	50

The silica xerogel sample was immersed in 50 ml SBF in a polyethylene bottle covered with a tight lid. Alternately, 5 ml sample or the whole medium was withdrawn from each flask and replaced immediately with fresh medium. Three parallel samples were used.

was determined by measuring the absorbance at 405 nm. The experiments were performed according the route illustrated in Scheme 1 comprising the steps:

1. Heparin + ATIII → heparin/ATIII complex
2. Heparin/ATIII + thrombin (excess) → heparin/ATIII/thrombin + residual thrombin
3. Chromozym TH → peptide + pNA (measured at 405 nm)

Platelet poor plasma (57 µl) was diluted with Tris buffer solution (245 µl, pH 8.3) and 150 µl sample solution in a 5 ml test tube. The test tubes were stirred and incubated at 37°C for 3 min. 150 µl of thrombin solution (8 IU/ml, Sigma T-7009, St. Louis, MO, USA) was added, mixed and incubated for additional 60 s at 37°C. Then 150 µl Ghromozym TH solution (1.13 mM, previously heated to 37°C, Tos-Gly-Pro-Arg-pNA, Boehringer Mannheim, Mannheim, Germany) was added, mixed and incubated for 310 s at 37°C. The reaction was stopped by adding 450 µl of 50 % acetic acid. The samples were analyzed spectrophotometrically at 405 nm using a Shimadzu UV-1601 spectrophotometer. Heparin standards between 0.2 and 1.0 IU/ml were done as samples. The relative biological activity was calculated by comparing the thrombin neutralization of immobilized heparin with that of free heparin. The rate of increase in absorbance at 405 nm due to the appearance of the chromophore, p-nitroaniline, is linearly and inversely related to the effective activity by means of standard curve.

Results

The test results of certain formulations prepared are shown in Table 3.

Heparin release from the different formulations examined occurred during the dissolution of the matrix. At the end of dissolution period (96 h), 10 % of the matrix in the tested formulations was dissolved, measured by silica content, and the same amount of heparin was released, suggesting that the heparin release is controlled by matrix erosion. Heparin release from a formulation containing 1 wt-% of heparin in the sol was identical to the rate of the matrix dissolution. Heparin release from the matrix was measured with toluidine blue method and according to silica xerogel matrix erosion studies. This implies that drug release may be described as a process that is controlled mainly by erosion of the matrix. In addition, the porosity of the matrix have an noticeable effect on the dissolution process. Especially in the case of small molecules, drug release is combined process of diffusion and matrix erosion.

Effect of catalyst

A model formulation containing 1 wt % heparin in the sol in order to investigate the influence of the used catalyst in the hydrolysis process on the dissolution rate of heparin. Silica xerogel monoliths were prepared using either acetic acid or nitric acid catalyst. At the pH 2.5 the hydrolysis step was faster while nitric acid was used, 45 - 60 min, than the one carried out by using acetic acid, 5 hours. According to the literature (Brinker & Scherer), the rate and extent of the hydrolysis reaction is most influenced by the strength and concentration of the acid catalyst. All strong acid behaved similarly, whereas weaker acid required longer reaction times to achieve the same extent of the reaction. The reaction rate with weaker acid can be accelerated by increasing the used reaction temperature.

and the heparin concentration did not have an influence on the degradation rate of the matrix. These findings are in accordance with our previous paper (Ahola et al., 1999).

Effect of organomodified alkoxysilanes

The release rate of biologically active molecules can be influenced by chemical modification of the silica xerogel matrix (Böttcher et al., 1998). Incorporation of organomodified alkoxysilanes into hydrolysis step with TEOS results increasing hydrophobicity of the matrix and changes in porosity. In this study, modification of nitric acid catalyzed sol with co-hydrolysis of TEOS with METES, ETES or DMDES, was carried out. Partial substitution of TEOS with 10 or 25 mol-% of organomodified alkoxysilanes were used. This partial substitution results in more brittle materials. All monoliths were broken during dissolution period which of course have an effect on the release rate. The addition of 10 mol-% organomodified alkoxysilane into the sol did not have any significant effect on the release rate of heparin. The release of heparin was linear according to zero order kinetics from all formulations containing 10 mol-% of organomodified alkoxysilane. When the amount was increased to 25 mol-% , the release behaviour of heparin was better fitted to first order kinetics indicating diffusion controlled process. The release rate of the drug was increased 20 to 40 % when 25 mol-% ETES and DMDES were used. Another reason for the faster releasing rate, besides the brittle structure, can be decreasing possibility to form hydrogen bonds between silica network and heparin.

It will be appreciated that the methods of the present invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent for the specialist in the field that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

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CLAIMS

1. A composition for controlled release of a biologically active agent from a carrier, wherein the biologically active agent is heparin or a related biologically active acidic polysaccharide and that the carrier is a sol-gel derived silica xerogel, **characterized** in that the xerogel is derived from a tetraalkoxysilane such as tetraethoxysilane (TEOS) and that part of the tetraalkoxysilane is replaced by an organomodified alkoxysilane, preferably an alkylsubstituted alkoxysilane.

2. The composition according to claim 1, **characterized** in that the alkylsubstituted alkoxysilane is methyltriethoxysilane (METES), dimethyldiethoxysilane (DMDES) or ethyltriethoxysilane (ETES).

3. The composition according to claim 1 or 2, **characterized** in that the biologically active agent is heparin in an amount of 5 to 30 weight-% calculated on the air dried xerogel.

4. A method for the preparation of a composition according to any of the claims 1 to 3, **characterized** by the steps of

a) hydrolysing an alkoxysilane and an organomodified alkoxysilane in the presence of a catalyst,

b) optionally adjusting the pH to a value suitable for the biologically active agent,

c) adding the biologically active agent,

d) allowing the hydroxysilane to polymerize, and optionally

e) removing water and alcohol formed in the hydrolyzation from the mixture.

5. The method according to claim 4, **characterized** in that the alkoxysilane is a tetraalkoxysilane such as tetraethoxysilane (TEOS).

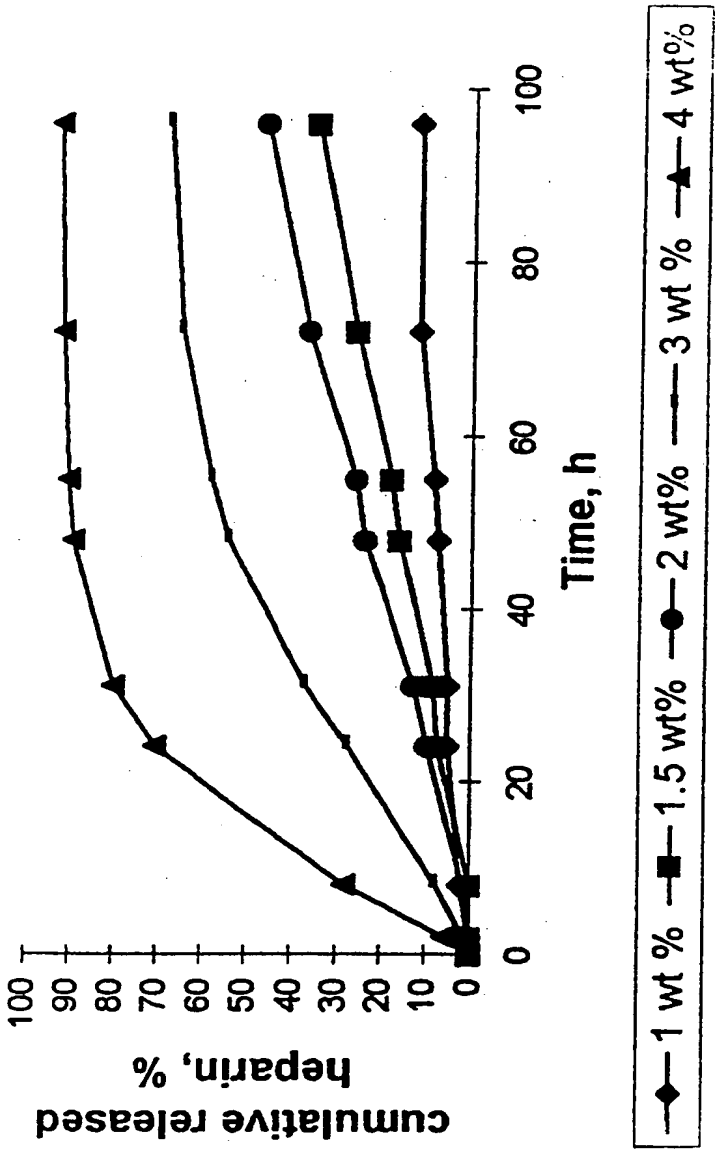
cumulative released heparin, %
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100
Time, h
1 wt %
1.5 wt%
2 wt%
3 wt %
4 wt%

FIG. 2

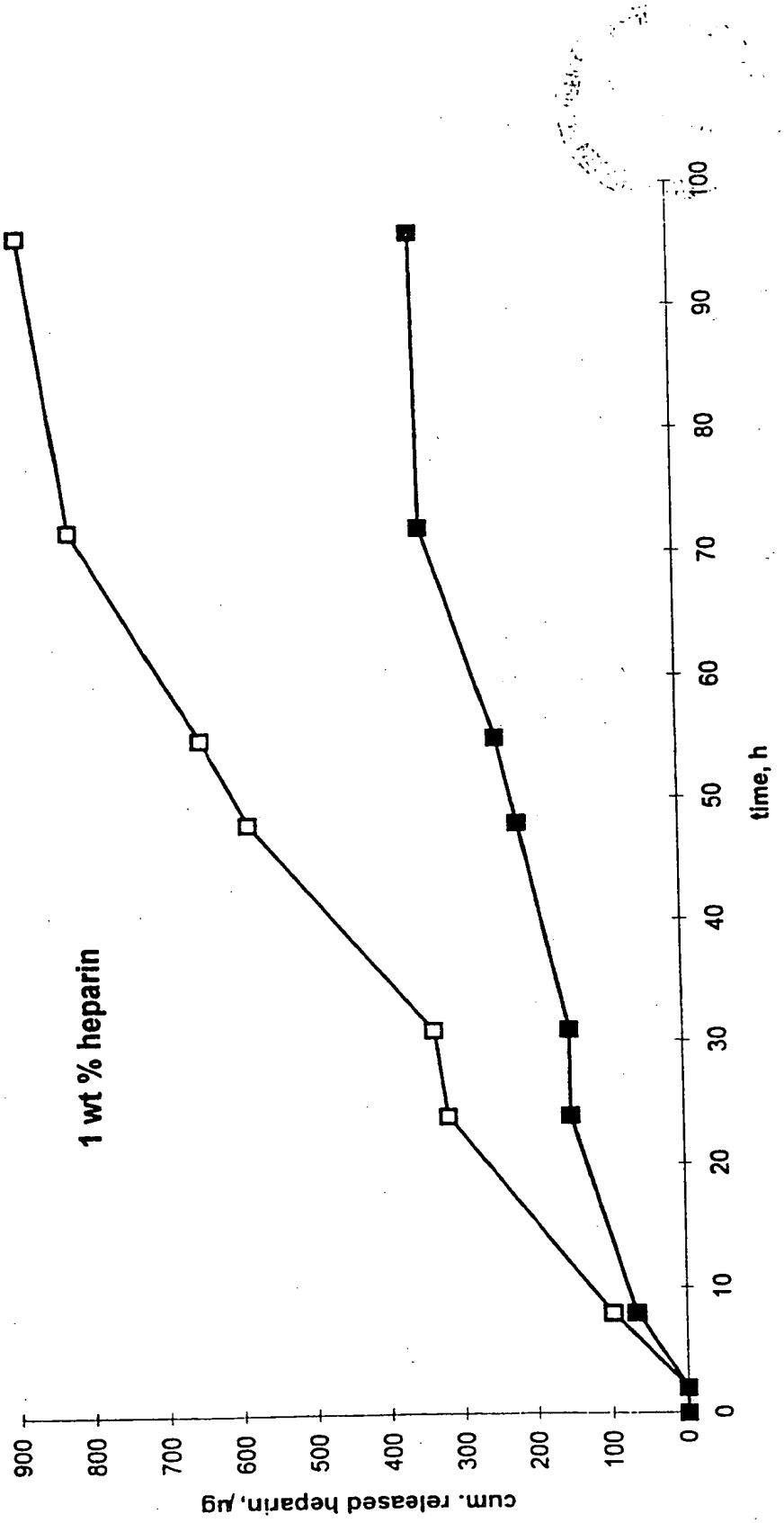

FIG. 1